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FINAL REPORT

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This document is the final report on research activities supported by AFOSR AGREEMENT number FA9550-06-1-0188, entitled Processing, Properties and Morphology of Optical Limiting Silk Membranes. It covers the 8-month period from 3/1/06, to its end on 11/30/06. This grant supported the exploratory research in optical limiting silk membranes and coatings to protect DOD sensors from strong IR radiation. The topics investigated in this research program are listed in Section II of the Table of Contents below.

I TECHNICAL RESULTS

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TECHNICAL RESULTS

1. Preparation of thin films of silk doped with GFP

1.1 Preparation of thin films of regenerated *B. Mori* silk doped with GFP

Cocoons were degummed to remove the glue-like sericin proteins. Degumming was accomplished by boiling cocoons in aqueous solution of 0.02M Na₂CO₃ for 30 min. The degummed silk was then rinsed thoroughly with water and allowed to dry at room temperature. The extracted silk was dissolved in 12M LiBr solution at 60°C. This solution was dialyzed against water for 3 days. The final concentration of aqueous silk solution was about 4 wt%. Regenerated silk solutions and GFP solutions were mixed in the ratios shown in Table 2. Then, they were cast on glass slide and allowed to dry at room temperature.

Table 2 Composition of mixed regenerated *B. Mori* silk/GFP Solutions

Sample #	Regenerated silk solution (g)/ GFP Solution (g)
1	1/1
2	2/1
3	4/1
4	10/1

1.2 Preparation of thin films of gland silk doped with GFP

The silk glands of *B. mori* were dissected just before spinning and rinsed with deionized water. The membrane was removed from the gland and the sericin was washed from the surface of the exposed fibroin by successive rinses with distilled water. Thereafter, the fibroin in the middle division (M) of the gland was divided into the posterior section (Mp) toward the synthesis area, the middle section (Mm), and the

anterior section (Ma) toward the spinneret. The middle section (Mm) was slowly dissolved to about 1wt% in deionized water. The silk and GFP solutions were mixed in the ratios shown in Table 1. The mixed solutions were cast on glass slides and allowed to dry at room temperature.

Table 1 Composition of the mixed fibroin/GFP Solutions

Sample #	Type of Fibroin	Fibroin Solution(g)/ GFP Solution(g)
2	Mm	4/1
3	Mm	20/1

1.3 Preparation of thin films of regenerated spider silk doped with GFP

The spider silks of *Argiope aurantia* were dissolved in 12M LiBr solution at 60°C. This solution was dialyzed against water for 3 days. The final concentration of aqueous silk solution was about 0.4 wt%. Regenerated spider silk solutions and GFP solutions were mixed in the ratios shown in Table 3. Then, they were cast on glass slide and allowed to dry at room temperature.

Table 3 Composition of mixed regenerated spider silk/GFP Solutions

Sample #	Regenerated spider silk solution (g)/ GFP solution (g)
4	2/1
5	5/1
6	10/1

2. Morphology of thin films of silk doped with GFP

2.1 Morphology of thin films of regenerated *B. Mori* silk doped with GFP

Films cast from regenerated *B. Mori* silk doped with GFP were observed using optical, fluorescent and atomic force microscope. Film with low concentration of GFP (silk:GFP 10:1) is transparent as shown in Figure 1. When this film was viewed under UV light, it fluoresced (Figure 1b). Neither the glass substrate nor the undoped silk film exhibited such fluorescence. A higher magnification UV image of 10:1 regenerated *B. Mori* silk:GFP, (Figure 2a), showed uniformly distribution of GFP. There was no evidence of phase separation at 500x magnification as shown in Figure 2a. Phase separations in film of regenerated *B. Mori* silk doped with GFP appeared when concentration of GFP increased as shown in Figure 2b-d. Size of phase domain increased as concentration of GFP increased. The GFP rich phase in 4:1 and 2:1 Silk:GFP films were indicated by bright green spots as shown in Figure 2b and Figure 2c respectively. The AFM image of 4:1 silk:GFP in Figure 3b showed the circular raised regions which have an average diameter of 0.3 μ m. Similar feature was shown in Figure 3c for 2:1 silk:GFP with diameter range from a few hundreds nanometer to a few microns. These circular raised regions in both 4:1 and 2:1 silk:GFP films represented the GFP rich phase. The irregular spots on 1:1 silk:GFP film (Figure 2d) appeared to be islands with holes in the middle as shown in AFM image (Figure 3d). Surface of transparent film of 10:1 silk:GFP film appeared to be wrinkle as shown in AFM image (Figure 3a).

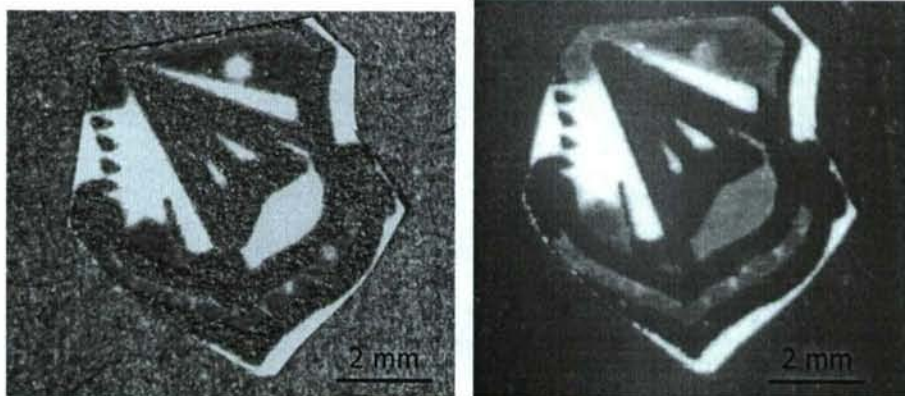


Figure 1 Optical image of films of regenerated *B. Mori* Silk doped with GFP over Air Force emblem under (a) visible light and (b) UV light . Ratio of silk solution to GFP solution is 10 to 1.

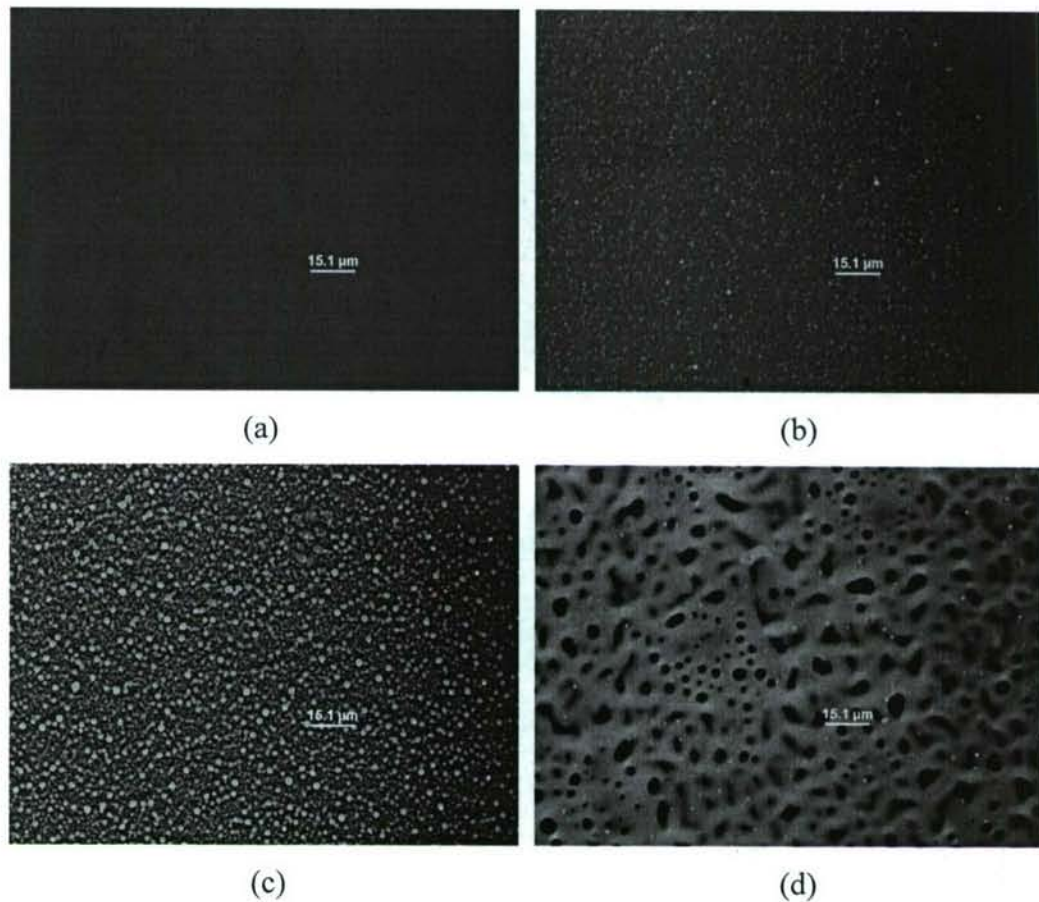


Figure 2 Optical images of films of regenerated *B. Mori* Silk doped with GFP under reflected UV light (epifluorescence) (a) regenerated *B. Mori* Silk solution: GFP solution 10:1, (b) regenerated *B. Mori* Silk solution: GFP solution 4:1, (c) regenerated *B. Mori* Silk solution: GFP solution 2:1, and (d) regenerated *B. Mori* Silk solution: GFP solution 1:1.

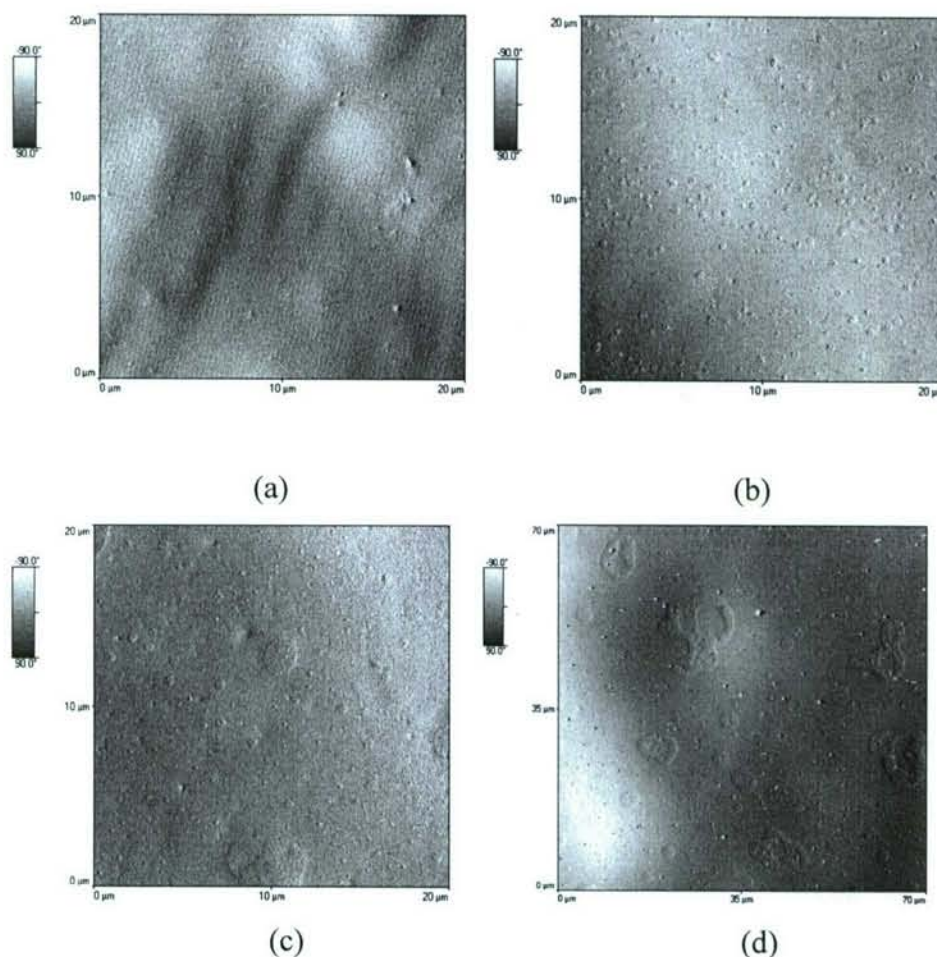


Figure 3 AFM images of films of regenerated *B. Mori* Silk doped with GFP under reflected UV light (epifluorescence) (a) regenerated *B. Mori* Silk solution: GFP solution 10:1, (b) regenerated *B. Mori* Silk solution: GFP solution 4:1, (c) regenerated *B. Mori* Silk solution: GFP solution 2:1, and (d) regenerated *B. Mori* Silk solution: GFP solution 1:1.

2.2 Morphology of thin films of gland silk doped with GFP

Preparation of thin films of gland silks doped with GFP has been extended from the work done under AFOSR AGREEMENT number F49620-03-1-0169. Morphology of gland silk doped with GFP was observed using fluorescent microscopy. Phase behavior of gland silk doped with GFP film was similar to that of regenerated *B. Mori* Silk doped with GFP film. There was no phase separation in film with low

concentration of GFP (silk:GFP 10:1) as shown in Figure 4a. Phase separation occurred when concentration of GFP increased (silk:GFP 4:1) as shown in Figure 4b. However, phase domain in film of gland silk doped with GFP was considerably bigger with higher variation in size than phase domain in film of regenerated *B. Mori* Silk doped with GFP film. Size of phase separation in film of gland silk doped with GFP ranged from a few microns to several tens microns.

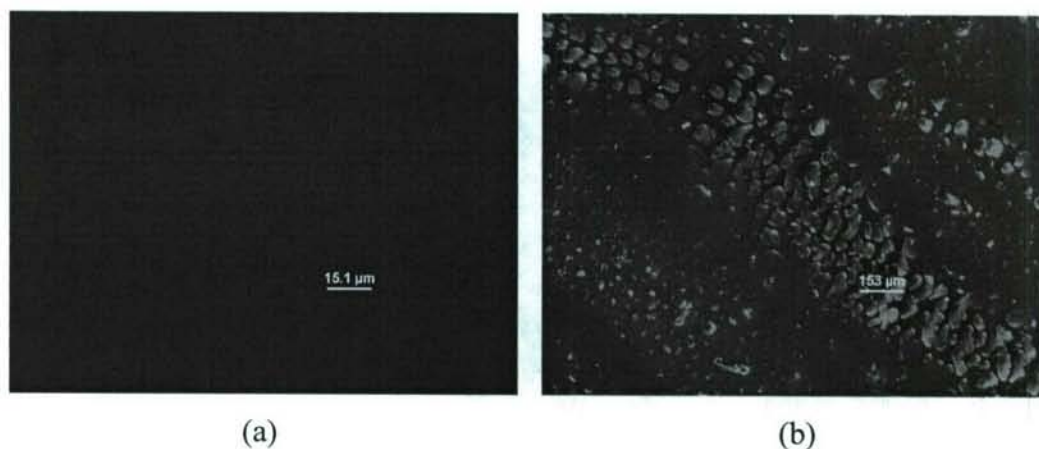


Figure 4 Optical images of films of gland silk (Mm) doped with GFP under reflected UV light (epifluorescence) (a) Gland silk solution: GFP solution 20:1 and (b) Gland silk solution: GFP solution 4:1.

2.3 Morphology of thin films of regenerated spider silk doped with GFP

Concentration of regenerated spider silk solution was lower than concentration of regenerated *B. Mori* solution as a result of solubility limitation of spider silk fiber in LiBr. Ratio of regenerated spider silk to GFP was about ten times lower than ratio of regenerated *B. Mori* solution to GFP since the limit amount of spider silk available. Nonetheless, phase behavior of regenerated spider silk doped with GFP film was similar to that of regenerated *B. Mori* Silk doped with GFP film. Phase separation in regenerated spider silk film is more evident when concentration of GFP was increased as shown in Figure 5. As concentration of GFP was increased to 4:1 silk:GFP (Figure 5c), leaf-like morphology was observed. The leaf-like feature was also observed in GFP as shown in Figure 6.

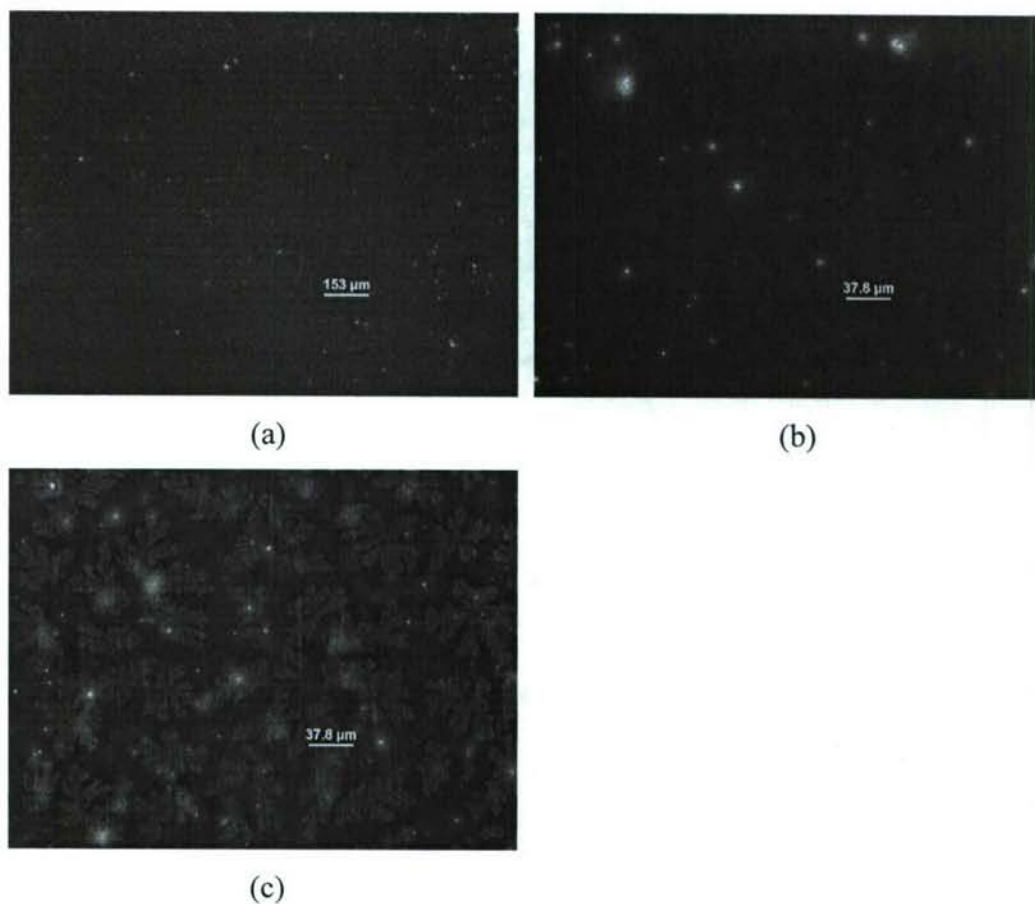


Figure 5 Optical images of films of regenerated spider silk doped with GFP under reflected UV light (epifluorescence) (a) regenerated spider silk solution: GFP solution 10:1, (b) regenerated spider silk solution: GFP solution 5:1, and (c) regenerated spider silk solution: GFP solution 2:1.

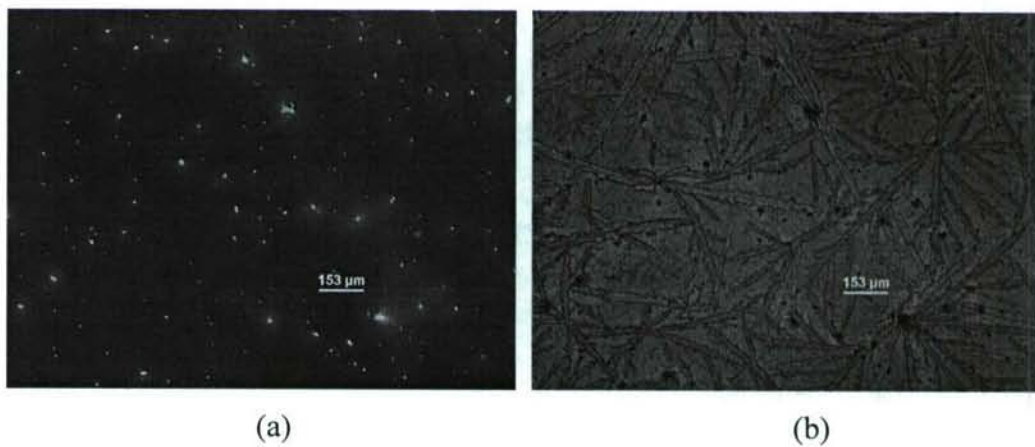


Figure 6 Optical images of GFP under (a) reflected UV light and (b) reflected visible light. These two picture were taken at the same area.

3. Nanoindentation test of silk doped with GFP

The two mechanical properties most frequently measured using nanoindentation technique are the elastic modulus (E) and the hardness (H). A typical quasi-static load-displacement curve is shown in Figure 7. As the load is increased, the indenter sinks into the material due to both elastic and plastic deformation, which results in the formation of a hardness impression conforming to shape of indenter. If the load is held constant, the indenter continues to sink into the material due to time-dependent deformation (creep). When the indenter is unloaded, the material recovers by a process that is primarily elastic. In Figure 7, h_{max} represents the displacement at peak load, P_{max} . h_c is contact depth and is defined as the depth of indenter in contact with the sample under load. S is initial unloading contact stiffness. The hardness value was derived from:

$$H = \frac{P_{max}}{A}$$

where A is projected area of indentation. The elastic modulus was calculated from initial unloading slope according to:

$$E = \left(\frac{\pi}{A} \right)^{1/2} \frac{S}{2}$$

where $S = \Delta P / \Delta h$ is initial unloading stiffness.

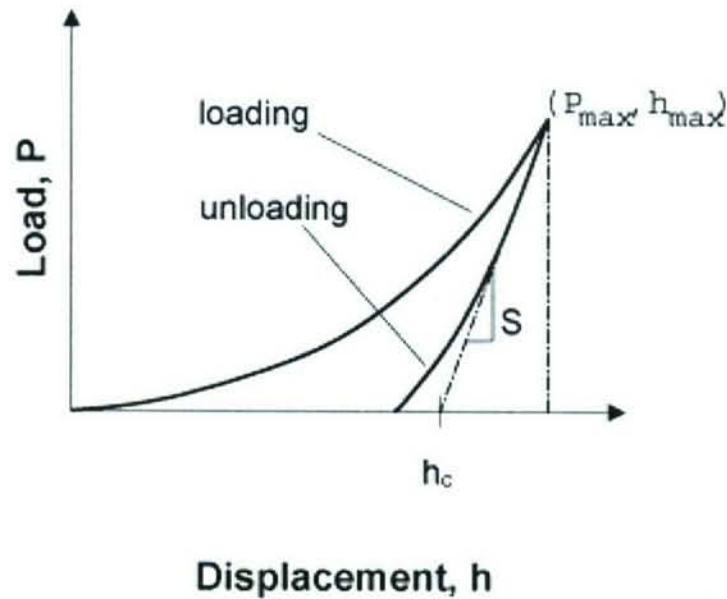


Figure 7 Schematic plot of a typical load-displacement curve

The continuous stiffness measurement (CSM) provides a significant improvement in nanoindentation test. The CSM allows a continuous measure of stiffness (S) along the loading curve, not just at the point of initial unloading as in the conventional measurement. This is accomplished by superimposing a small oscillation on the primary loading signal and analyzing the resulting response of the system by means of a frequency-specific amplifier. The CSM technique makes the continuous measurement of mechanical properties of material in one experiment without the need for discrete unloading cycle. The measurements can be made at very small penetration depth. Therefore, this technique is suitable for mechanical properties measurement of thin films. Figure 8 showed schematic of the CSM loading cycle. The displacement response of the indenter at the excitation frequency and the phase angle between the two are measured continuously as a function of depth. Solving for the in-phase and out-of-phase portions of the response results in explicit determination of the contact stiffness, S , as a continuous function of depth.

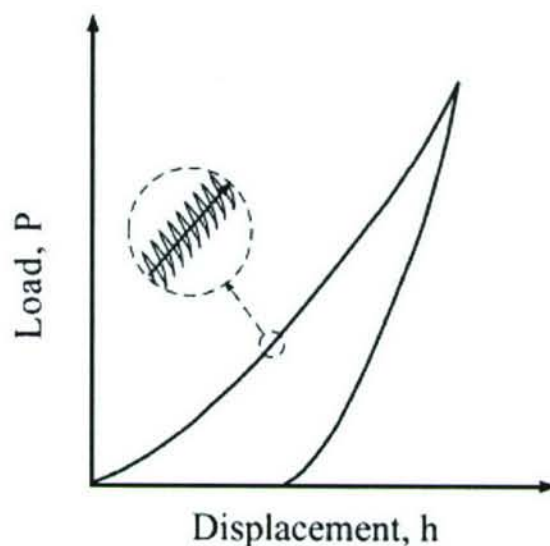
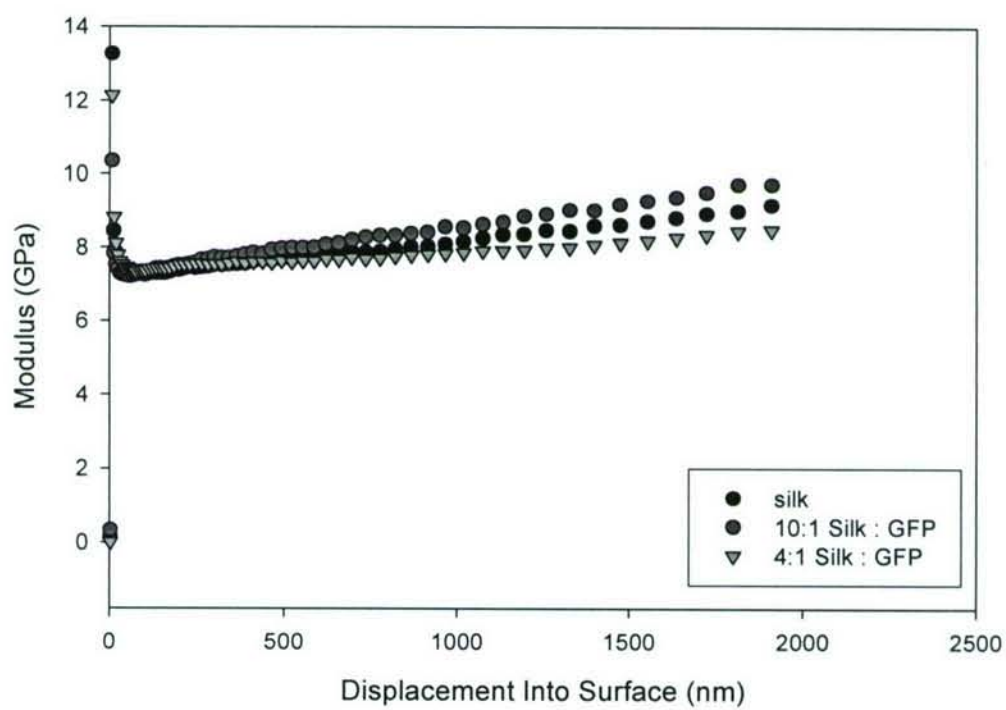
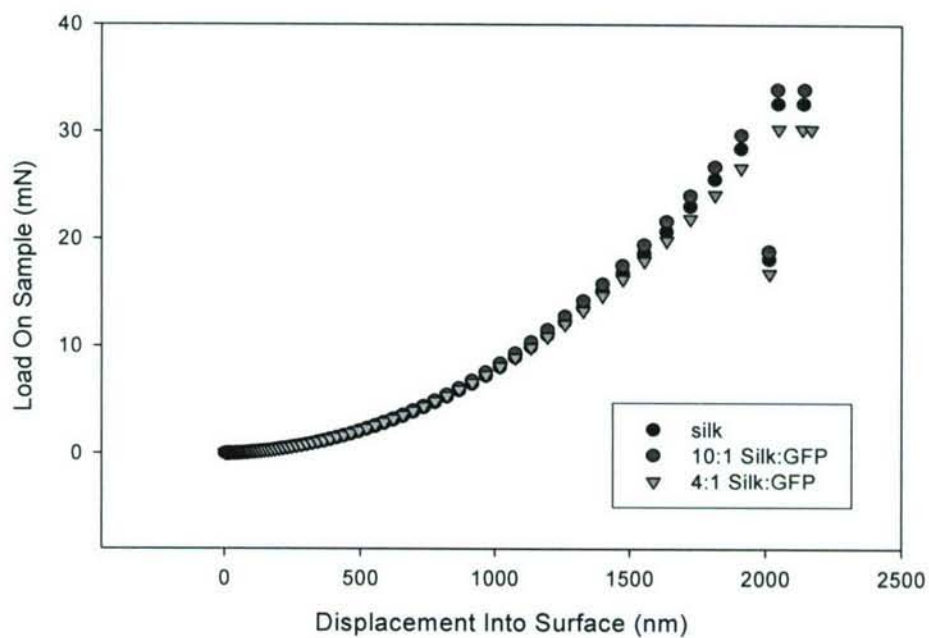
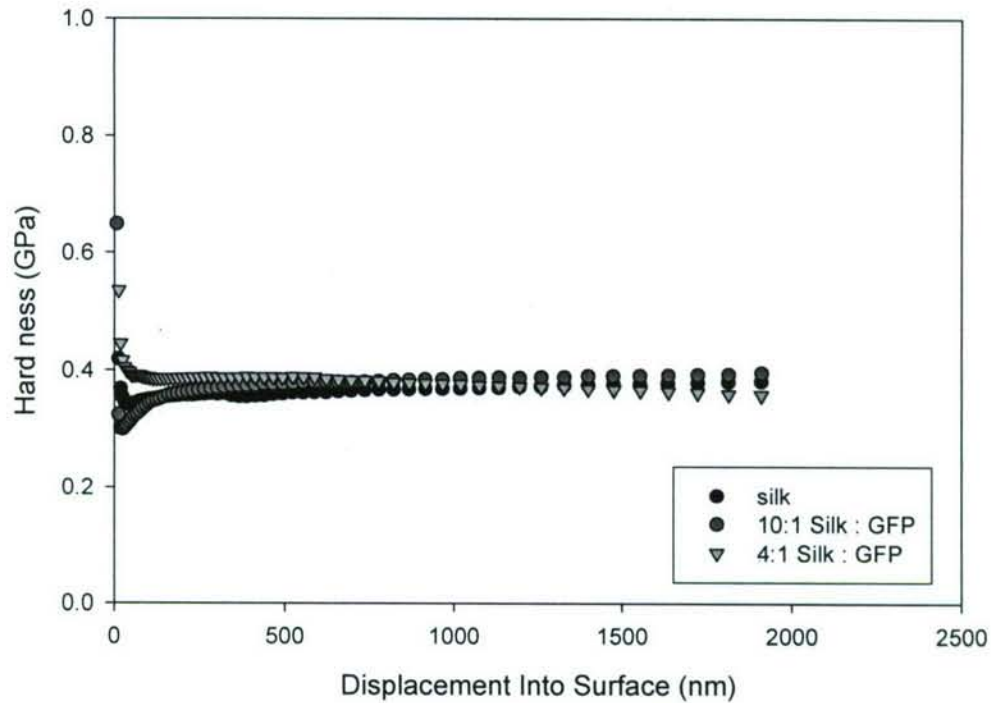


Figure 8 Schematic of the CSM loading cycle

The elastic properties of regenerated silk doped with GFP films were measured by using Nano Indenter XP system placed in vibration-free isolated cabinet. The hardness (H) and the elastic modulus (E) of regenerated silk doped with GFP films were measured with Berkovich three-sided pyramid diamond indenter with a nominal angle (defined by the tip axis and faces) equal to 65.3° . The CSM was used to measure hardness (H) and elastic modulus (E), with a load resolution of 50 nN and 0.01 nm, respectively. In all CSM tests, about 15 points of indents on different area were averaged to determine E and H values.





4. Nonlinear fractional transmission test of silk doped with GFP

The nonlinear transmission of the films was measured by placing each sample near the focal region of a 100cm lens, and measuring the incident and transmitted energies of pulses from an amplified Ti:S laser (Clark-MXR CPA-2010, 775nm, 140fs). Incident pulse energies ranged between 60 and 500 μ J at a repetition rate of 500Hz. The incident intensity was then modulated by changing both the pulse energy and the position of the sample in the laser focus. Transmission was measured as a function of increasing input intensity until a small damaged spot was observed on the film surface (damage of the glass substrate was not observed). Measurements were made at several positions on each sample to check for signal variations due to non-uniformity of thickness and GFP concentration throughout each film. Upconverted fluorescence was also observed, but nonlinear generation from the substrate appeared negligible.

Figure 9 showed fractional transmission versus incident intensity for film of regenerated *B. Mori* Silk solution: GFP solution 10:1. The nonlinear nature of the film's transmission was clearly evidenced by its systematic decrease with increasing incident

light intensity. There was some variation in the values caused by variations in film thickness and concentration of GFP in each area of film.

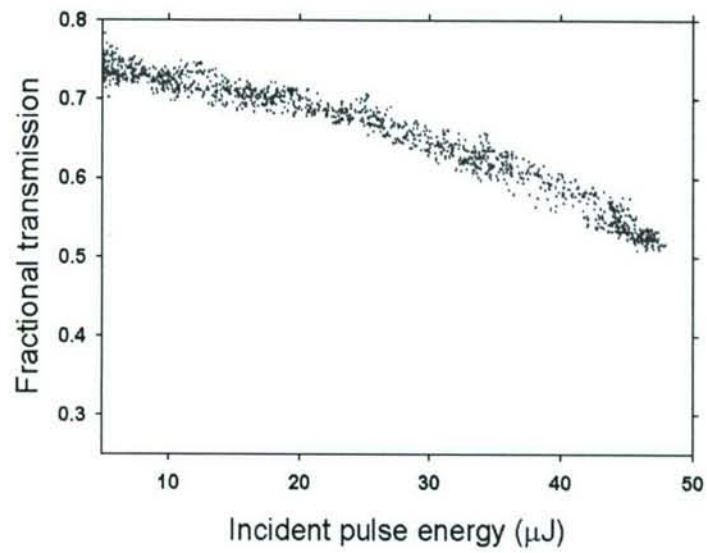


Figure 9 Nonlinear fractional transmission for film of regenerated *B. Mori* Silk solution: GFP solution 10:1.

OTHER ACTIVITIES SUPPORT BY GRANT

A. Collaborations

- Developing project with Prof. Todd Blackledge at the University of Akron to make membranes from a range of spider silks with different compositions as well as to make tensile measurements on silk nanofibers.
- Developing project with Prof. Shing-Chung Wong at the University of Akron on the mechanical properties of cast silk/GFP nanocomposite membranes.

B. Consultative and Advisory Functions to Materials Lab at WPAFB

- Collaborating with ML on preparing electrospun silk/PEO/GFP nanocomposite membranes.

PRESENTATIONS AND PUBLICATIONS SUPPORTED BY GRANT

A. Publications (Peered Reviews)

1. Electrospun *Bombyx mori* Gland Silk, S. Putthanarat, R.K. Eby, W. Kataphinan, S. Jones, R. Naik, D.H. Reneker, B.L. Farmer, Polymer, In Press, (2005).
2. Axial Deformation of Dragline Silk of *N. Clavipes*, D.V. Mahoney, R.K. Eby, In Press Journal of Macromolecular Science, (2005).

B. Participation/Presentations at Meetings, Conferences, Seminars, etc

1. American Physical Society (APS) Meeting, Contributed, "Influence of Green Fluorescent Protein (GFP) Nanoparticles on the Optical and Mechanical Properties of Silk in Bio-Nanocomposites with Photonic Properties", March 2006.